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ABSTRACTS FROM THE ORIGINAL PAPERS.

Studies On the Amylases of Different Origins for Alcohol Brewing I.

Quantitative Comparision of Amylo-liquefying, Amylo-dextrinizing and Amylo-saccharifying Enzymes of Germinated and Ungerminated Cereals, and Molds.

By KOKICHI OSHIMA and SHINICHI ITAYA.

The purpose of this study is to ascertain the best amylolytic agent and condition for alcohol brewing by comparing many kinds of amylolytic enzymes, especially of molds, such as Aspergillus oryzae and Asp. awamori, which have special use in Japanese breweries. Also the effect of mixing of mold amylase and malt amylase as the saccharifying agent.

A. Enzymic samples experimented were as follows:—

a. Purified enzymes (mostly alcohol precipitant) of germinated barley, soya beans, Aspergillus oryzae and Asp. awamori.

b. Molds—Asp. oryzae, Cohn (3 strains), Asp. awamori, Nakazawa, Rhyzopus delemar (Boid) Wehm. et Hanz. and Rh. japonicus Saito. These were cultured on cooked wheat bran, which was considered the best medium to produce the strongest amylase. The enzymic activities were tested with the water extract.

c. Germinated and ungerminated cereals—barley (Hordenum sativum, Jensen) (3 kinds), wheat (Triticum vulgare, Vill), rice (Oryza sativa, L.) (2 kinds), oats (Avena sativa, L.) (2 kinds), rye (Secale cereale, L.), soya beans (Glycine soya, Benth), barnyard grass (38 kinds) (Panicum Crus-Galli, L.)

B. Experimental methods :—

a. Amylo-liquefying enzyme. With Osima-Itaya's method (11), by the change of viscosity of starch paste, digested at 40°C for 30 minutes.

b. Amylo-dextrinizing enzyme. With a little modification of Wohlgemuth's method (18), by iodine reaction of soluble starch solution, digested at 40°C for 30 minutes.

c. Amylo-saccharifying enzyme. With Oshima's method (9), by reducing power of produced sugar from digested soluble starch solution at 40°C fot 30 minutes.

All enzymic activities (=quantities) were measured for the unit of one gram of original dry cereal or dry culture medium.

C. Optimum reactions and quantitative estimations of the three kinds of amylases of the samples. The amylolytic activities were tested in every sample but only the strongest sample of each species is here described.

Samples	Amylo-liquefying enzyme		Amylo-dextrinizing enzyme		Amylo-saccharifying enzyme	
	optimum P_H	quantity	optimum P_H	quantity	optimum P_H	quantity
Purified enzyme of Asp. oryzae	4.5–5.2	36,000	5.2	50,000	5.2	4,040
〃 germ. barley	4.5	20,000	4.5	33,340	4.5–6.2	6,100
〃 ungerm soya beans	5.2	800	5.2	260	4.8	21,000
〃 Asp. awamori	4.5	10,800	4.5–5.2	20,000	5.2	2,740
Asp. oryzae	4.5	1,000	4.5	833	5.2–5.8	206
Asp. awamori	4.5	388	4.5	250	4.8–5.2	55
Rhyzopus japonicus	5.2	400	5.2	250	5.2	194
〃 delemar	4.8	50	4.8	67	4.8	11
Germinated barley	5.2	125	5.2	555	4.5–4.8	166
〃 barn yard grass	4.5–5.2	400	5.2	500	4.5–5.2	41
〃 wheat	6.5	360	—	—	5.2–5.8	576
〃 oats	5.2–6.5	160	5.2	500	5.2	41
〃 rice	—	—	—	—	5.2	6
Ungerminated rye	4.5	13	4.5	55	4.5	132
〃 rice	—	—	—	—	5.2	0.1
〃 barn yard grass	—	—	—	—	—	0
〃 soya beans	—	—	—	—	4.8	295
〃 barley	—	—	—	—	—	0
〃 wheat	6.5	1.4	—	—	5.2	118
〃 oats	—	—	—	—	—	0

D. Conclusion and discussion.

1. The optimum reaction of the actions of three amylases from various samples

were almost same, i. e., PH 4.5–5.8. Only exception was wheat (ungerminated), which had PH 5.2–5.8 for amylo–saccharase, and PH 6.5 for amylo–liquefying enzyme.

2. Reaction range of the considerable activity was in general PH 3.0–7.0, as exception wheat amylase acted at basic side a little more.

3. The amylo–liquefying and amylo–dextrinizing enzymes variated with almost same ratio for every sample. On the contrary, the amylo–saccharifying activity variated independently with the two above.

4. To compare the amylolytic activities of different samples, purified enzymes must be omitted, as they differ according to their preparation method. The strongest amylo–liquefying enzyme was of Asp. oryzae, the next was germinated barn yard grass, Asp. awamori, germinated wheat. The strongest amylo–saccharifying enzyme was in the order of Asp. oryzae, germinated barley and ungerminated rye.

5. Ungerminated soya beans, germinated wheat, germinated barley and ungerminated rye had stronger amylo–saccharifying than amylo–liquefying enzyme comparing with Asp. oryzae, Asp. awamori, Rh. delemar and Rh. japonicus. On the contrary, the last group had stronger amylo–liquefying enzyme than amylo–saccharifying enzyme. What would be the effect of mixing the two groups? This will be presented in next report.

6. The comparison some kinds of germinated barley showed the amylo–saccharifying activities between 146–340. The length of the cotyledon over 1 cm. had little change on enzymic activities.

7. 38 kinds of germinated barn yard grass showed the amylo–saccharifying activities between 6.4–40.8 and the average was 16.5.

8. Comparison between glutinous and common rice showed not much difference for their amylo–saccharifying activity. Both activities increased about ten times by germination. Still the strongest activity was only 6.0.

9. Ungerminated rice, barley, oats and barn yard grass had little amylase, but ungerminated soya beans, wheat and rye had quite strong amylase. By germination, wheat increased its amylo–saccharifying activity 5 times, whereas its amylo–liquefying activity 250 times.

10. Oats of several kinds showed similarly the saccharifying activity about one fourth of that of germinated barley, but the liquefying activity was stronger than the latter.

11. Three strains of Asp. oryzae were compared their optimum reaction and activity of 3 amylases. The optimum reaction for three amylases remained the same. The ratio of the activity of three amylases was almost same and they showed much stronger amylo-liquefying activity than germinated cereals. The amylo-saccharifying activities were 68–206. Other experiments (10) showed that the activity of many strains of Asp. oryzae variated greatly. The strongest amylo-saccharifying activity was 260, which is probably the strongest amylase produced among molds.

On Sulphur Containing Amino-acids. IV.

The Determination of Cystine in Urine.

By Yuzuru Okuda and Yuraku Nishijima.

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A new method for the determination of cystine in urine is described. It is an application of the iodine method recently published by one of the authors (Okuda: J. Chem. Soc. Japan, **45**, 18, 1924.······Okuda and Motomura: This jounal, in press).

The method is directly applicable in the normal urine and also in the presence of sugar, but indirectly in the presence of much protein and hydrogen sulphide. Most of the protein should be previously removed by boiling with a few drops of acetic acid and hydrogen sulphide by aeration.

The Method:—The following solutions are required,···(1) About 5% KI aqueous solusion. (2) Exactly 4% HCl. (3) Exactly 2% HCl. (4) Exactly 20% NaOH. (5) Cystine solution, which contains 1 mg, cystine in 1 c.c. of 2% HCl. (6), M/1000 KIO_3, which is prepared by dissolving 0.214 g. of pure KIO_3 in 1 liter of exactly 2% HCl. For standardization of this solutson for cystine, dissolve 0.2 g of cystine in 50 c.c. of about 5 % HCl, add a few decigrams of zinc dust, leave it for 30 minutes at room temperature. Filter, wash and make it up to 100 c.c. Take 1 c.c. of the filtrate in a dry bottle or a test tube, mix with 19 c.c. of exactly 2 % HCl, 5 c.c. of 5 % KI and 5 c.c. of exactly 4 % HCl, and then titrate with the iodate solution until a yellow colour is produced. Insert immediately a thermometer in the mixed liquid to know the temperature in which the experiment has been finished. The quantity of the iodate solution corresponds to 2 mg. of cystine, at that temperature. It is convenient to repeat the same experiments several times in different temperatures, and to get a table or a curve showing the relation between the temperature and the required volume of the iodate solution.

Procedure:—Take 50 c.c. of urine, add 10 c.c. of the cystine solution, boil for 1 minute with about 0.5 g. of the best charcoal and then cool for 10 minutes. Filter with a small Buchner funnel, wash thrice with 5 c.c. of water. Add about 0.5 g. of fine zinc dust and 15 c.c. of about 20 % HCl. Leave it for 30 minutes at room temperature for reduction. Filter again with a small Buchner funnel, wash twice with 5 c.c. of water. Make the filtrate up to 100 c.c. with water. Take 1 c.c. of the solution for the determination of the concentration of HCl in it. To 90 c.c. of the residual portion of the solution, add a calculated quantity of 20% NaOH to make it into a solution containing exactly 2 % of free HCl. After ascertaining by titration that the solution contains exactly 2 % HCl, take 20 c.c. of the solution, mix with 5 c.c. of 5 % KI and 5 c.c. of 4% HCl, and then titrate with the standard iodate solution until the yellow color produced remains for 1 minute. Read the temperature of the liquid. Calculate the amount of cystine in the 20 c.c. of the solution, using the table or the curve previously obtained. Subtract 10 mg. from the total amount (mg) of cystine obtained, then the rest is the quantity of cystine in 50 c.c. of the original urine.

If the original sample contains some cysteine, the results obtained as above express the sum of cystine and cysteine as cystine.

The separate determination of cystine and cysteine is easily accomplished, titrating a sample solution before and after the reduction.

It the special case of urine which contains too much thiocyanate, this should be removed. The original paper describes a method to deal with such a case. But the case is very rare.

Looney's method (Jour. Biol. Chem., **54**, 171, 1922.) for the determination of cystine in urine was compared with the present method. The former gave much higher results, especially in the case where less uric acid is present in the urine.

On the Waxy Substance Coating on the Cuticle of the Bamboo Joints.

K. KAWAKAMI.

Phyllostachys, Edulis A., et, C. Rivière, the largest stem species of the bamboo grow in Japan, has a white powder-like waxy substance on the cuticle of its joints. This substance is scraped off from the bamboo nodes with finger-tips and tested. It melts at 80°-81°C., and does not dissolve in ethyl alcohol, methyl alcohol, carbon tetrachloride, and ether at the room temperature, and also scarcely soluble even being heated, however, somewhat soluble in chloroform and carbon disulphide at the ordinary temperature, and more readily when heated. It does not give the Liebermann's cholesterol reaction like those waxes of grape or apple.

The most part of the soluble substance in cold chloroform consists of a higher paraffin, which being isolated in a pure state by distillation at a diminished pressure. The substance thus obtained seems to be a hydrocarbon of methane series, having a melting point of 63°-64°C, being identical with that of nonacosane; and by its analysis and the molecular weight the substance is found to be very likely same as nonacosane:

Analysis :		found	(1)	C = 85.12%	H = 14.67%
			(2)	C = 84.65 〃	H = 14.65 〃
Nonacosane	$C_{29}H_{60}$	Calc.		C = 85.29 〃	H = 14.71 〃
Molecular weight		found	(1)	395	
			(2)	385	
	$C_{29}H_{60}$	Calc.		408	

The insoluble part of the wax in chloroform at room temperature is saponified with 5% Na-alcoholate, and $CaCl_2$ is added as to make a Ca-soap. Then the alcohol is evaporated off, and the residue is extracted with hot chloroform in oder to remove the wax-alcohol and hydrocarbon present. After the resulted substance is decomposed by boiling with dilute HCl, a certain fatty acid is formed, and it is decolorized, and recrystallized from ethyl alcohol, and finally is crystallized in a fine needle form. Its melting point is 78°C., which is almost identical with that of melissic acid, which melts at 78.5°-79°C. The result of analysis, and the molecular weight are as follows.

Analysis :	found	(1)	C = 79.06%	H = 13.20%
		(2)	C = 79.76 〃	H = 13.14 〃
Mellissic acid $C_{30}H_{60}O_2$	Calc.		C = 79.65 〃	H = 13.27 〃
Molecular weight	found	(1)	432	
		(2)	453	
$C_{30}H_{60}O_2$	Calc.		453	

The above extracted portion of the saponified substance is separated into the two parts:—the wax alcohol and the hydrocarbon, by the method of Leys, (J. Pharm. et chim. **5**, 277, 1912), and the part of wax alcohol is several times recrystallized from ethyl alcohol, and finally is made in a needle crystalline form, which has a silky lustre, melting at 85°–87°C. The author believes this substance should be melissyl alcohol, which melts at 86°–88°C. The figures of the analysis, and determination of the molecular weight are shown below.

Analysis :	found	C = 82.19%	H = 13.97%
Melissyl alcohol $C_{30}H_{61}OH$	Calc.	C = 82.19 〃	H = 14.16 〃

The result of analysis of the acetate of the alcohol is as following.

Analysis :	found	C = 80.02%	H = 13.57%
$C_{30}H_{61}COOCH_3$	Calc.	C = 80.00 〃	H = 13.33 〃

Besides those three compounds, the portion that is soluble in cold chloroform contains a crystalline substance which can be dissolved in acetic acid, and so it is obtained by extracting it with 80 % acetic acid. When the extract is concentrated to about half its volume the substance is crystallized out itself. It is soluble in chloroform, hot ethyl alcohol, and acetic acid. It contains a certain mineral matter, and melts at 240°–250°C with decomposition. The yield of the substance is so poor that the author could not study further more.

The mother liquid contains some resin which is soluble in most organic solvents and can be burnt readily giving a characteristic smell resembling that of terpentine.

Quantities of those substances were as follows.

Nonaccsane	$C_{29}H_{60}$	55%
Melissic acid and Melissyl alcohol ($C_{30}H_{60}O_2$ + $C_{30}H_{61}OH$)		20 〃
unknown crystalline substance		2 〃
resin		4 〃

On Vitamine C in Japanese Sand Pear (Pyrus serotina Rehder) and Kaki-Fruits (Diospyros Kaki L.)

By YASUO IWASAKI.

The author found that the vitamine C content in the flesh of Japanese sand pear, Chōjūro (a horticultural variety), was very poor, but when its rind and flesh were compared, the rind part was a little richer.

The method used by the author for the experimental scurvy was the following: growing guinea pigs were fed on the mixture of equal volume of wheat bran and rolled oats at libitum, a daily ration of 40 c.c. of milk autoclaved for an hour at 120°C. and water given freely after the animals had taken the milk. After the animals had shown obviously the scorbutic signs, several amounts of the juice of the above named fruits to be tested were given to them in addition to the above foods. The juice of each fruit was freshly prepared every day before giving it, being grated and squeezed the fruit through cotton-cloth.

45 c.c. of the juice of pears, not containing the rind part were added every day, but this could not cure the scurvy, and the animal died on the 26th day from the time the juice had been added.

The juice from the flesh containing rind part showed better antiscorbutic potency, because the animals which took 41 c.c. of it every day, became gradually cured from the disease, and after 40 days they restored their health utterly. One animal which took 34 c.c. of the juice every day, showed a slight symptom of scurvy yet.

The juice of the ripe Kaki-fruits Fuyū (a horticultural variety) was given to two animals, 10 c.c. per day for 30 days. The animals cured completely during the period. So it can be said that the Kaki juice has a very good antiscorbutic power, but its minimum dose which can cure scurvy can not be determined from these experiments.